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Reference Intervals of Routine Coagulation Assays During the Pregnancy and Puerperium Period

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Background: Significant changes occur in the coagulation and fibrinolytic systems during pregnancy and puerperium in the plasma levels. However, reference ranges based on healthy people are not optimal for informing clinical decisions during the pregnancy and puerperium. Therefore, it is essential to explore coagulation assays' reference ranges during the pregnancy and puerperium. *Methods:* Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (Fib.), and D-dimer were all measured according to the manufacturer's specifications and laboratory standard operating procedure of the STA-R evolution coagulation analyzer. A total of 11,601 women were enrolled in this study. *Results:* The reference ranges for PT, APTT, TT, Fib., and D-dimer in nonpregnancy period were 10.87–13.76 s, 29.22–44.61 s, 15.39–20.15 s, 1.59–3.97 g/l, and 0–0.56 mg/l, respectively. In early-pregnancy period, the ranges were 11.14–14.07 s, 29.97–44.69 s, 14.92–19.03 s, 1.98–4.13 g/l, and 0–1.67 mg/l, respectively. In midpregnancy period, the ranges were 9.98–12.84 s, 28.53–40.70 s, 13.51–19.82 s, 2.63–5.19 g/l, and 0–2.81 mg/l, respectively. In late-pregnancy period, the ranges were 9.48–12.58 s, 28.61–40.80 s, 14.10–19.61 s, 2.80–5.56 g/l, and 0–27.08 mg/l, respectively. In puerperium period, the ranges were 10.85–13.72 s, 30.51–43.02 s, 15.31–19.64 s, 1.14–5.07 g/l, and 1.27–4.85 mg/l, respectively. *Conclusion:* We presented reference intervals for coagulation assays from the nonpregnancy to puerperium period that can be adopted in other laboratories after further validation. J. Clin. Lab. Anal. **30**:912–917, 2016. © 2016 Wiley Periodicals, Inc.

Key words: coagulation assays; pregnancy; puerperium; reference intervals; STA-R

INTRODUCTION

Significant changes occur in the coagulation and fibrinolytic systems during pregnancy, delivery, and puerperium in the plasma levels (1). Abnormal coagulation and fibrinolytic systems could lead to high morbidity or mortality in the mother and fetus (2). The incidence of venous thromboembolism is approximately 0.76 to 1.72 per 1,000 pregnancies, which is about 4–50 times higher than that in nonpregnant women, especially in the late-pregnancy and puerperium periods (2, 3). Elevated markers of coagulation and fibrinolytic systems' activation indicate increased thrombin generation and increased fibrinolysis following fibrinogen–fibrin conversion throughout the pregnancy (4). In previous studies, the level of coagulation factors II, V, X, XI, XII and antithrombin; protein C; activated partial thromboplastin time (APTT); and prothrombin time (PT) remained unchanged essentially during the pregnancy, delivery, and postpartum and were within the nonpregnant reference intervals. However, levels of coagulation factors VII, VIII, IX, fibrinogen (Fib.), and D-dimer increased markedly (5, 6). In another study, PT, APTT, and thrombin time (TT) were decreased significantly, but Fib. level rose with the gestational age, which referred to a hypercoagulable

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TABLE 1. Characteristics of the Study Population

Groups	Number	Median age (years)	SD	Minimum age (years)	Maximum age (years)	Median gestational age (weeks)
Nonpregnancy	659	30	8.40	16	57	—
Early pregnancy[a]	4,377	29	6.41	16	47	10
Midpregnancy	762	29	4.97	16	48	22
Late pregnancy	5,067	29	4.73	17	47	36
Postpartum	736	31	6.82	20	45	—
Total	11,601	29	11.26	16	57	—

[a]Early pregnancy: ≤12 weeks, midpregnancy: 13–27 weeks, and late pregnancy: 28–42 weeks.

state (7). These changes were considered physiological for maintaining placental function during the pregnancy and preventing significant blood loss during delivery, but may predispose to thrombosis and placental vascular complications at the same time.

Reference intervals were the basic framework of laboratory testing and were also used by clinical doctors as important comparative measurements to decide whether patients required further examinations. Some gestational reference intervals have been reported previously; however, differences existed among the results of the same specimen owing to different laboratory conditions and different experimental instruments and methods. Likewise, differences also existed to some extent among the physiological indexes due to different environments and different locations. Therefore, it was necessary to establish the pregnancy and puerperium period references for coagulation assays in our laboratory. In this study, some changes occurred during the normal pregnancy and postpartum, which were investigated to establish adequate reference intervals for important coagulation parameters. Five coagulation assays of plasma levels were included: PT, Fib., APPT, TT, and D-dimer.

MATERIALS AND METHODS

Study Subject Recruitment and Sample Collection

A total of 11,601 female who were treated as outpatients and from hospitalization were enrolled from August 2012 to April 2015 in the Second Affiliated Hospital of Zhengzhou University. The participants were included using the following selection criteria: singleton pregnancy (normal pregnant women), diastolic blood pressure <90 mmHg, no thromboembolic disease, no bleeding disorders, and no hematuria and proteinuria. Exclusion criteria were the history of clinical diseases and drugs that could affect coagulation factor levels. Among the participants, there were 659 nonpregnant (control group), 4,377 early-pregnancy (≤12 weeks), 762 midpregnancy (13–27 weeks), 5,067 late-pregnancy (28–42 weeks), and 736 postpartum women (Table 1). The median ages in the nonpregnancy, early-pregnancy, midpregnancy, late-pregnancy, and postpartum groups were 30, 29, 29, 29, and 29, respectively.

The research protocol was approved by the institutional Ethics Committee of the Bioscience department of Zhengzhou University. All the participants signed an individual informed consent after receiving a detailed explanation of the research; and the data were processed respecting the confidentiality of the participants.

The plasma samples were collected by venipuncture into vacuum tubes containing 0.109 mol/l trisodium citrate. The blood tubes were centrifuged immediately at 3,000 × *g* for 15 min at room temperature. D-dimer assays were measured with the latex-based immunoturbidimetry and other coagulation assays were measured with magnetic bead coagulation. All tests were completed on the STA-R evolution coagulation analyzer with identical reagents (Diagnostica Stago) within 2 h.

Coagulation Assays' Measurement

All tests were performed according to the manufacturer's specifications and laboratory standard operating procedure of the instrument (Diagnostica Stago). During the testing, a routine assay model was chosen: if the concentration of D-dimer was more than 4 mg/l, the sample was diluted fivefold and retested; if the concentration of D-dimer in the diluted sample was still more than 4 mg/l, the sample was further diluted tenfold and retested.

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 statistical package for windows. The results were reported as mean values depending on the type of distribution. The reference interval of coagulation assays was presented with 95% confidence intervals. Dixon's algorithm was used to remove the outliers. The nonparametric analysis was performed to compare the results from the groups examined. All tests were two-sided and interpreted as being significant at $P \leq 0.05$.

TABLE 2. Reference Intervals of PT, Fib., APTT, TT, and D-dimer for the Nonpregnant, Pregnant, and Postpartum Women

	Nonpregnancy			Early pregnancy			Midpregnancy			Late pregnancy			Postpartum		
	n	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
PT (s)	659	12.31	10.87–13.76	4377	12.60	11.14–14.07	762	11.41	9.98–12.84	5067	11.03	9.48–12.58	736	12.29	10.85–13.72
Fib. (g/l)	659	2.78	1.59–3.97	4377	3.05	1.98–4.13	762	3.91	2.63–5.19	5067	4.18	2.80–5.56	736	3.10	1.14–5.07
APTT (s)	659	36.92	29.22–44.61	4377	37.33	29.97–44.69	762	34.62	28.53–40.70	5067	34.71	28.61–40.80	736	36.77	30.51–43.02
TT (s)	659	17.77	15.39–20.15	4377	16.97	14.92–19.03	762	16.66	13.51–19.82	5067	16.85	14.10–19.61	736	17.47	15.31–19.64
D-dimer (mg/l)	659	0.26	0–0.56	4377	0.61	0–1.67	762	1.11	0–2.81	5067	3.36	0–27.08	736	3.06	1.27–4.85

RESULTS

Five Coagulation Assays' Values During the Nonpregnancy Period

The reference intervals of PT, Fib., APTT, TT, and D-dimer for healthy people from Diagnostica Stago instrument were 11–14 s, 2–4 g/l, 30–46 s, 14–21 s, and 0–0.5 mg/l, respectively. In this study, the mean values of PT, Fib., APTT, TT, and D-dimer in the nonpregnancy control group were 12.31 s, 2.78 g/l, 36.92 s, 17.77 s, and 0.26 mg/L, respectively (Table 2). The 95% confidence interval values of the five coagulation assays in the nonpregnant control group were 10.87–13.6 s, 1.59–3.97 g/l, 29.22–44.61 s, 15.39–20.15 s, and 0–0.56 mg/l, respectively. There was no difference between the nonpregnant control group and healthy people.

Changes in the Five Coagulation Assays During the Pregnancy Period

The 95% confidence interval values of PT, APTT, TT, Fib., and D-dimer in the early-pregnancy period were 11.14–14.07 s, 29.97–44.69 s, 14.92–19.03 s, 1.98–4.13 g/l, and 0–1.67 mg/l, respectively. In the midpregnancy period, the ranges were 9.98–12.84 s, 28.53–40.70 s, 13.51–19.82 s, 2.63–5.19 g/l, and 0–2.81 mg/l, respectively. In the late-pregnancy period, the ranges were 9.48–12.58 s, 28.61–40.80 s, 14.10–19.61 s, 2.80–5.56 g/l, and 0–27.08 mg/l, respectively. The mean values of PT in the early-pregnancy, midpregnancy, and late-pregnancy groups were 12.6, 11.41, and 11.03 s, respectively (Table 2). PT level increased in early pregnancy. However, PT levels progressively decreased throughout the midpregnancy and late pregnancy (Fig. 1A). The mean APTT values in the three pregnancy groups were 37.33, 34.62, and 34.71 s. Changes in APTT were the same as PT in the three pregnancy groups (Fig. 1D). Fib. and D-dimer concentrations gradually increased throughout the pregnancy (Fig. 1C and F). The mean values of Fib. were 3.05, 3.91, and 4.18 g/l in the three pregnancy groups. However, TT levels progressively decreased throughout the whole pregnancy (Fig. 1E). The mean values of TT in the early-pregnancy, midpregnancy, and late-pregnancy groups were 16.97, 16.66, and 16.85 s, respectively (Table 2). We found no difference among the three pregnancy groups. Compared with the nonpregnancy women, the mean values of PT, TT, APTT, Fib., and D-dimer in all pregnant women were significantly different ($P < 0.01$).

Changes in the Five Coagulation Assays During the Puerperium Period

The 95% confidence interval values of PT, APTT, TT, Fib., and D-dimer in the puerperium period were 10.85–13.72 s, 30.51–43.02 s, 15.31–19.64 s, 1.14–5.07 g/l, and 1.27–4.85 mg/l, respectively. The mean values of PT, TT, and APTT during the puerperium period were 12.29, 17.47, and 36.77 s (Table 2), which were all higher than pregnant women with statistically significant differences. Fib. and D-dimer showed statistically significant fall in the levels from the pregnancy to puerperium period (Fig. 1).

DISCUSSION

The clinical laboratory results were usually compared to their reference intervals before the doctors made medical diagnoses and physiological assessments. Thus, it was an important task for clinical laboratory to provide reliable and accurate reference intervals. In order to establish reference intervals, a large healthy population was required. However, it was very difficult to obtain completely healthy normal, pregnant, and puerperium women. Thus, hospital-based reference values were ideal for interpretation that it was the same as other studies (7). In this study, a total of 11,601 women were obtained from a population of outpatient and hospitalized normal, pregnant, and puerperium women in our hospital.

Many studies have investigated changes in the coagulation and fibrinolytic systems during the pregnancy and puerperium (8, 9). However, no study had a sufficient number of patients in changes of PT, TT, APTT,

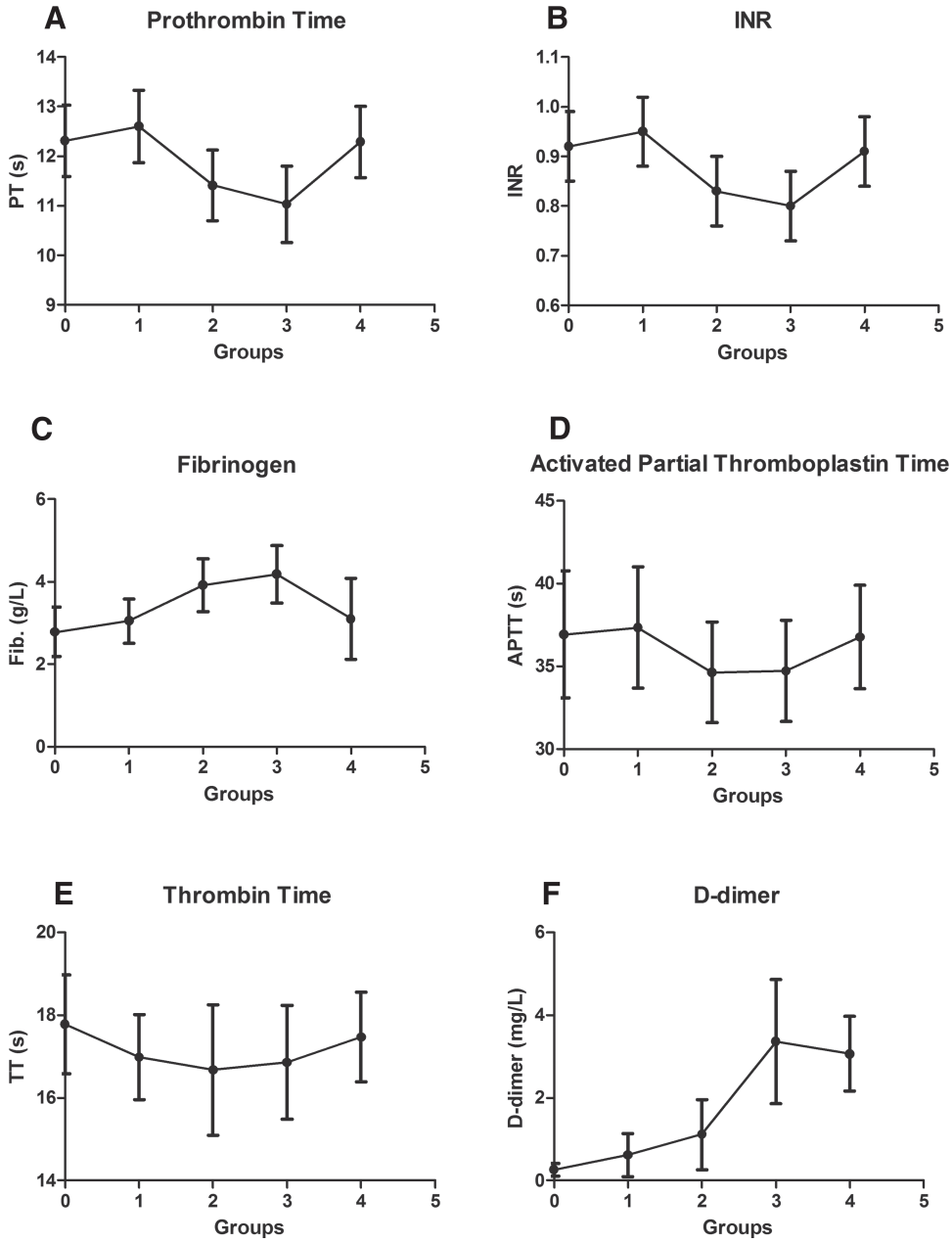

Fig. 1. Evolution of the PT, INR, APTT, TT, Fib., and D-dimer levels in different periods. At the horizontal axis: 0, nonpregnancy; 1, early pregnancy; 2, midpregnancy; 3, late pregnancy; and 4, postpartum.

Fib., and D-dimer from the nonpregnancy to puerperium period. Some studies were small, with 30–50 patients in each group and no specific reference values for pregnant women calculated (1, 10). Few studies have investigated the changes in the coagulation and fibrinolytic systems immediately after delivery (11). After delivery, both the blood coagulation and fibrinolytic systems are activated or increased for at least 2 weeks although the inhibitory capacity of both systems is increased simultaneously (12). In this study, we found significant changes in the five coagulation assays during the pregnancy and puerperium (Table 2).

We used the magnetic bead coagulation on the STA-R evolution coagulation analyzer, but other studies may differ (7, 13). Magnetic bead coagulation was a new method, which can avoid the interferences with hemolysis, jaundice, and lipemia. So, our results would be more accurate to re-act the real situation of the patients.

This study is unique in that it is the first reference interval study in China during the pregnancy and puerperium

period using the STA-R evolution coagulation analyzer. Our results contribute with further evidence of biochemical changes in coagulation during the pregnancy and puerperium. The changes found in our study were consistent with previous studies. The five coagulation assays were all analyzed by the same STA-R evolution and identical reagents. A D-dimer below the cut-off is a valuable negative predictor of active thrombosis.

Changes in the mean values of the five coagulation assays during the pregnancy significantly differed from nonpregnant women. PT, TT, and APTT were shortened progressively during the pregnancy period, which was in accordance with previous reports (9, 14). Compared with the nonpregnant conventional levels, Fib. and mean concentrations were higher in each examined gestational week. This was in agreement with other studies and these findings were to be taken into account when evaluating these parameters in pregnant women (6–8, 15). However, the calculated reference ranges differed between the studies and examined populations. In a previous report, D-dimer had higher reference values and Fib. had lower (16). In another study, with the same reagents as we used, the reference ranges for Fib. and D-dimer were highly comparable with our results (17). These differences suggested that laboratories should establish their own reference intervals and cut-off points also in pregnancy in order to help the clinician's work, although data from such studies can be exemplary in certain clinical situations.

In the puerperium period, we examined the progressive changes of the five coagulation assays. The mean values of PT, TT, and APTT were all higher than in the pregnant women. Fib. and D-dimer statistically significantly decreased. Few studies had reported the changes of coagulation assays in puerperium period (18, 19). However, the use of different analytical platforms renders them unique as to require method-specific reference intervals. The five coagulation assays' reference intervals in our study were different compared with previous studies (20, 21).

CONCLUSION

We presented reference intervals of the five coagulation assays from the nonpregnancy to pregnancy period by obtaining data from a large sample. It is important for understanding the profound changes that take place in the coagulation system during the pregnancy and puerperium. Thus, our study confirms the fact that profound changes in blood coagulation take place during a normal pregnancy and puerperium and stresses the need for adequate reference values during the pregnancy and puerperium. According to the CLSI and IFCC guidelines, our reference ranges can be universally adopted in other clinical laboratories after appropriate validation.

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CONFLICT OF INTEREST

All the authors declare that they have no competing interests.

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